CHAPTER TWELVE

The Identity of the Transforming Substance

The story of the advances made by Dawson and Sia (*in vitro* transformation, 1933), Avery, MacLeod and McCarty (identification of the transforming substance, 1944) has been told many times. I shall deal here with only one aspect of the work—the progressive characterization of the transforming agent—since it was this aspect which led to the first evidence for the genetic role of DNA. It also serves to throw into relief the very different approaches of the Lamarckian Griffith together with those who confirmed his work and adopted his interpretation on the one hand, and on the other the group under Avery who demanded and obtained a precise chemical account of bacterial transformation.

Naturally the early workers thought the agent of transformation must in some way be dependent upon a protein. They had Griffith's evidence that it was thermolabile. Perhaps, wrote Dawson, "the S vaccine disintegrating in the animal tissues supplies a suitable pabulum . . ." (1928, 121). But as it could not be the specific soluble substance itself, perhaps it was a co-ferment which, together with the synthesizing enzymes of the receptor cell, made possible the production of the carbohydrate capsular substance. Neufeld and Levinthal thought: "Possibly a precursor of the specific carbohydrate or a carbohydrate bound to a protein" was necessary (1929, 340). When Dawson and Sia had succeeded in producing transformation *in vitro* they realized that the transforming substance bore a "striking resemblance to the 'antigenic specific substance described by Day" (1931, 709). H. B. Day at the Institute of Pathology, St. Mary's Hospital, London, had shown that the antigenic specific substance must consist of two portions, one thermostable which reacted with immune serum, the other thermolabile and susceptible to the attack of bacterial enzymes. This latter portion provoked the production of antibodies (Day, 1930). Evidence for such a twofold character to the bacterial antigen of course goes back to earlier work (Zinsser and Parker, 1923, Avery and Heidelberger, 1923) when the theromolabile portion was described as a nucleoprotein. Griffith's immediate successors, however, did not pursue this identification. They had enough to do trying to achieve an

experimental situation in which transformation could be reproduced consistently and *in vitro*.

When Wilhelm Baurhenn in Heidelberg confirmed the *in vitro* transformation achieved by Dawson and Sia he supported Dawson's "co-ferment" idea for the agent responsible (1932, 91). Lionel Alloway, who prepared the way for Avery, MacLeod and McCarty by his achievement of cell-free *in vitro* transformation, avoided committing himself on the chemical identity of the agent. It could not, he reasoned, be the polysaccharide alone unless this specific soluble substance was present "in a different physical state, or in combination with some other substance . . ." (1933, 276). Later, we are told, he favoured the protein-containing full capsular antigen (Hotchkiss, 1966, 184).

Alloway had used sodium desoxycholate (bile salt) to liberate the contents of the pneumococcal cells and these, when extracted with salt solution, could be passed through Berkefeld filters and yet remain as active in transformation as the intact pneumococcal cells. Now when Alloway slowly added this bacterial salt solution to 500 cc of chilled absolute alcohol "a thick syrupy precipitate formed . . ." (1933, 266). This must surely have consisted of fibrous, biologically active DNA (Hotchkiss, 1965, 5), but "it was *not* implicit in general experience, in Alloway's time, that a thick, stringy alcohol precipitate meant DNA: some mucus, linear polymers and what was called 'renosin' (not *known* to contain DNA) behaved like that" (Hotchkiss, 1972). At the time, then, this biologically active stringy precipitate would most likely have been considered a protein or nucleoprotein but definitely not a polysaccharide, since the latter was not precipitated by alcohol.

Three years after Alloway described this alcohol-precipitated fibrous substance Avery is reported to have said that "the transforming agent could hardly be carbohydrate, did not match very well with protein, and wistfully suggested that it might be a nucleic acid!" (Hotchkiss, 1965, 5). Certainly this solubility in salt solution and precipitation by alcohol looked more like that of a nucleic acid than of a protein, and then there were the repeated reports of the isolation of an antigenic, though not type specific, nucleoprotein, by salt extraction and precipitation with the dropwise addition of acetic acid (Avery and Morgan, 1925). And had not Dubos shown that RNase destroyed the antigenicity of dead encapsulated pneumococci? (see Chapter 6). A biological function in the host-bacterium relationships was thus associated with a nucleic acid of the yeast type. Hence, in 1936 Avery either did not concern himself with the question of the type of nucleic acid involved or he would perhaps have favoured the yeast-type. But this is just speculation and: "In fact, it was not like him to settle on any single substance until there was strong evidence for it" (McCarty, 1972). We must remember, too, that by the time Hotchkiss arrived at the Rockefeller (1935) Avery was

telling his intimates of the separation of the protein-containing antigen from the transforming activity (Hotchkiss, 1966, 184). Even so, it is doubtful that thymus-type nucleic acid was seriously considered, especially since it had not been extracted from pneumococci, whereas nucleic acid susceptible to RNase was extracted not long thereafter (Thompson and Dubos, 1938).

Characterization by Enzymology

The Rockefeller led the world in enzymology, so what more obvious and appropriate strategy could there be than to pursue the identity of the transforming agent by studying the enzymatic destruction of its activity? In 1946 McCarty and Avery had this to say of their approach:

> The enzymatic analysis was begun early in the course of the attempts to determine the nature of the transforming substance. Relatively unpurified pneumococcal extracts were subjected to enzymatic activity in the hope that by this approach some clue might be obtained as to the identity of the biologically active constituent. Crystalline trypsin, chymotrypsin, and ribonuclease had no effect on the transforming substance, but it was found that certain crude enzyme preparations were able to bring about complete loss of transforming activity. When the possible importance of DNA was suggested by chemical fractionation, the experiments with crude enzyme preparations were extended to determine whether their ability to destroy the activity of the transforming principle could be correlated with any enzymatic action on authentic samples of DNA of non-bacterial origin.
>
> (McCarty and Avery, 1946a, 89)

From this passage, written only three years after the identification of the transforming substance as DNA, it is clear that the crystalline enzymes trypsin, chymotrypsin and ribonuclease were used early on. Their failure to inactivate the substance threw doubt on the possibility that it could be a protein. Such doubt would have been strengthened had they been able to use crystalline pepsin, but at the optimum pH for this general proteolytic enzyme the activity of the transforming agent was destroyed. By 1940 Moses Kunitz (at the Rockefeller) had prepared crystalline RNase and this, when used on the transforming substance, failed to inactivate it. By 1940, therefore, Avery and Colin MacLeod knew that their active substance was not RNA. At this point in the story Levene's contribution to nucleic acid enzymology came in handy. As we saw in Chapter 6, he and Schmidt had detected the presence of a DNA depolymerase in the secretion of the intestinal mucosa of a dog. Avery and MacLeod therefore used Levene's intestinal extract on the transforming substance. It did inactivate it. Perhaps, incredible though it might seem, the substance was thymonucleic acid!

Enzymatic studies were not alone in pointing in this direction. Avery and MacLeod, like Alloway before them, had precipitated the active substance using absolute ethyl alcohol. This was a well-known precipitant in several methods for the preparation of thymonucleic acid. Avery and MacLeod

therefore tried the technique of fractionation to increase the purity of their substance. Alcohol was added dropwise until at a critical concentration "varying from 0.8 to 1.0 volume of alcohol the active material separates out in the form of fibrous strands that wind themselves around the stirring rod" (Avery, MacLeod and McCarty, 1944, 143). When this fibrous substance was analysed it revealed the presence of phosphorus, it absorbed ultraviolet light at a maximum in the region 2600 Å, and it had a molecular weight of at least half a million. It gave a strong Dische reaction for DNA, but also a weak Bial reaction for RNA. When compared with the theoretical value for sodium thymonucleate the elementary analysis of the transforming substance agreed fairly well. By the time Maclyn McCarty joined in the work in 1942 the possibility that they were dealing with DNA was well founded, but by no means certain.

The sequel to the use of crude enzyme extracts on the transforming substance was their use on non-bacterial samples of DNA. Mirsky supplied them with such samples obtained from mammalian tissues and from fish sperm. Both were attacked.

Still not content, Avery encouraged McCarty to pursue the evidence yet further. He used crystalline RNase to remove RNA from the transforming substance and similarly the Dubos SIII enzyme to remove polysaccharide. After the publication of the great 1944 paper McCarty succeeded in purifying DNase to the point where it contained only traces of a proteolytic enzyme and no ribonuclease activity (McCarty, 1946a). This led in turn to an improved method for the preparation of the transforming substance (McCarty and Avery, 1946b), and to a striking demonstration of the power of this enzyme, even when present in minute quantities, to destroy the activity of the transforming substance permanently (McCarty and Avery, 1946a).

From this account it seems that the identity of the transforming substance was revealed slowly step by step. Just as there were no bold *a priori* ideas which experiment later validated, so there were no unfortunate and lengthy false trails which led nowhere. Illustrative of the way in which the problem of identity was solved, was the procedure of isolation. This came to incorporate stages for eliminating all the components whose inactivation or removal had early been shown to have no effect upon the activity of the transforming substance:

> the protein by the chloroform method, the capsular polysaccharide by digestion with a specific bacterial enzyme which hydrolyses it, the somatic polysaccharide by fractional alcohol precipitation, and ribonucleic acid either by enzymatic digestion with ribonuclease or by alcohol fractionation.
>
> (McCarty, 1946a)

All that has been said so far is in accord with the late Colin MacLeod's recollection:

> By the time McCarty joined us we were virtually certain of what we were dealing with, both on the basis of the methods of preparation, the physical–chemical properties, and the elementary analysis. Moreover, we had pretty good evidence that the enzyme which destroyed activity was DNase from a variety of lines of approach . . . Maclyn McCarty was a great help in tying things down and in getting further evidence that the enzyme was indeed DNase through the purification of that enzyme from pancreas.
>
> (MacLeod, 1967)

The Interpretation of Bacterial Transformation

All the writers before Avery, MacLeod and McCarty were inclined to accept Griffith's interpretation of transformation according to which the recipient cells had retained the power to elaborate the capsular polysaccharide of *several* serological types and needed only the specific *stimulus* of the transforming principle in order to produce any one of them. The serological types which resulted were of course determined by the type of the donor bacteria. Surprising as this may seem to us today, the truth of this assertion is based upon good evidence, as the following quotations show:

> The R form, therefore, probably results from attempts of S bacteria to adapt themselves to unfavourable environmental conditions. Once reduced to the S state the organisms potentially have the capacity to develop the S structure of any of the various specific S types.
>
> (Dawson, 1930, 143)

> The exact nature of the active material in these extracts still remains to be determined. That it acts as a specific stimulus to the R cells which have potentially the capacity of elaborating the capsular polysaccharides of any one of the several types of pneumococci seems clear.
>
> (Alloway, 1933, 277)

> But the decisive cause of the behaviour of the R form is present here . . . in the character of the variants themselves, for in them the process of degradation S to R has evidently not yet worked deeply, so that each specific stimulus to the formation of an heterologous S structure brings about its full regeneration as in the case of the residual *Anlage* of the type specific structure of the receptor R form.
>
> (Baurhenn, 1932, 84)

The 1944 Interpretation

Whatever has been said about the conservative stand taken by Avery and his colleagues in 1944 when they discussed the significance of their results (Pollock, 1970, 14; H. V. Wyatt, 1972, 87) it cannot be said that they followed the "Griffith line" described above. Certainly, they belonged to the empirical tradition. They set themselves a narrowly defined goal and pursued it single mindedly between 1940 and 1943. Experiments suggested ideas and these were in turn "controlled", to use a famous term of Claude Bernard, by further experiments, and they wrote:

> The major interest has centred on attempts to isolate the active principle from crude bacterial extracts and to identify if possible its chemical nature or at least to characterize it sufficiently to place it in a general group of known chemical substances.
>
> (Avery, MacLeod and McCarty, 1944, 138)

Now Dawson had left the Rockefeller in 1929 after confirming Griffith's work and had done the experiments with the Chinese scientist Richard Sia at Columbia University nearby. Alloway also left the Rockefeller after achieving transformation with cell-free extracts in 1932. When Colin MacLeod came two years later, one of his aims was to work on bacterial transformation—he had read Griffith's paper as a medical student, but no one was working on it at that time. Avery himself was away at a sanatorium for the second time, receiving treatment for what was later identified as hyperthyroidism. Meanwhile, MacLeod taught himself to isolate R forms of Type II pneumococcus and when Avery returned in the fall of 1935 they set to work using Alloway's *in vitro* cell-free system. From this point on it is clear that Avery was very much involved in the work. He was a bachelor and the Rockefeller was his second home. Like MacLeod, therefore, he would come in at week-ends to continue the work which involved regular 7 a.m. to midnight sessions when transforming extracts were being prepared (MacLeod, 1968).

Now what can we learn about Avery? Was he the original genius who stimulated all those around him and without whom immunochemistry at the Rockefeller would be unthinkable? Dubos wrote that he "was not as broadly informed a scholar as one would assume from his achievements and fame" (Dubos, 1956, 42). On the other hand he was convinced that biological specificity was determined by chemical specificity and he focused his attention on this to the exclusion of lesser matters. His greatest contribution, said Dubos, "was not so much a vision as a thread along which all the observations were organized. Avery was the person who went to the clinician, Dochez, and to the chemist, Heidelberger, . . . and crystallized all these fragments together" (Personal communication). "Nothing was of use to him unless it could be integrated into an intellectual picture" (Dubos, 1957). Those who came to work with him contributed their piece, and the picture took shape. This was the sense in which Avery acted as the leader. He did not organize and direct a team in the modern sense. He never suggested a topic to a young scientist, but all those who came to the Rockefeller hospital, especially those in the Pneumonia Service, "ended up in his office which was the ward kitchen up on the sixth floor, and Dr Avery would sit and tell them the laws of pneumococcus" (McCarty, 1968).

These talks were known as the "Red Seal Records"; they started off with background history, the long debate over the chemical basis of biological specificity. Could the specificity of an enzyme like Sumner's urease, or that of Northrop's pepsin, trypsin and chymotrypsin, really reside in the crystalline protein molecule? Similarly, could the reaction of pneumococcus with immune serum really depend upon the chemical composition of its polysaccharide coat? His account was interlaced with little aphorisms for the

experimenter such as: "It is lots of fun to blow bubbles, but it is wiser to prick them yourself before someone else tries to" (Avery, 1943). These talks led to wide reading by the young scientist and further discussions with Avery, out of which would emerge an idea for a research programme.

There is no escaping the conclusion that Avery had a profound influence on these young men. The admiration and loyalty expressed by those who worked with him is testimony to this. Just how much of the laboratory work in the transformation story he did himself does not therefore seem important. Nor should we be misled by his public caution into assuming that he was not privately confident about the success and significance of the work. Avery and MacLeod found the transformation system extremely difficult for the first three or four years, but despite temporary setbacks they "never felt they were not going to pull it off" (MacLeod, 1968). By the winter of 1941/42 they were "quite confident" that they were on the right track. It looked like a nucleic acid of the thymus type, but they had not narrowed down their focus to this class of compounds alone. Protein was still felt a possible claimant since some proteins were known to resist the action of trypsin and chymotrypsin and not to be destroyed by chloroform (McCarty, 1968).

In March 1943 Avery had reported at a meeting of the Trustees of the Rockefeller on the chemical identity of the transforming substance (Coburn, 1969, 628). That summer saw lengthy and agonizing discussions of the text of the famous paper which was eventually submitted to the *Journal of experimental medicine* in November 1943. According to McCarty there were no referees' reports on it. Peyton Rous "was carrying the complete editorial load himself, as he did for many years. (The names of Simon Flexner and Herbert Gasser appeared on the journal as Editors, but they delegated all authority to Rous)" (McCarty, 1970). Avery delivered it to Rous personally,

> and told his friend and colleague of some thirty years that he wanted him to review it just as he would a manuscript submitted by an unknown outsider. Two to three weeks later, I was present in Dr Avery's office when Dr Rous presented his comments and corrections to us verbally. He began by reminding us that Dr Avery had asked for a truly editorial review and stated that he had taken him at his word. The manuscript was covered with the pencilled notations and comments that were so characteristic of Dr Rous's editorial method. Some of these were small matters of wording or presentation, but there were also some more substantial suggestions. For example, we had included a quotation from J. B. Leathes in the discussion which was concerned with the speculation that nucleic acids might some day be found to surpass the proteins in importance. Rous pointed out that this, being merely a speculation, added little to the argument. It was deleted.
>
> (*Ibid.*)

Here, then, was one influence which served to dilute the impact of the discovery. We have already seen how many biologists were impressed with Leathes' address in the late 1930s (see p. 117). So with this unfortunate piece of prudery, the link between the old tradition of chemical individuality and the new discovery of the specificity of DNA was surpressed!

What did come out clearly in the discussion at the end of the '44 paper was that DNA was much more than a mere "midwife molecule", it was not just a structural frame, for it was "functionally active in determining the biochemical activities and specific characteristics of pneumococcal cells" (Avery, Macleod and McCarty, 1944, 155). Just how the determination was brought about was, of course, unknown, but Avery, MacLeod and McCarty suggested that the transforming principle "interacts with the R cell giving rise to a co-ordinated series of enzymatic reactions that culminate in the synthesis of the Type III capsular antigen" (*Ibid.*, 154).

But how was the DNA acting? Was it behaving as a gene as Burnet (1944) had suggested, or as a mutagen which caused mutation of the genetic material in the recipient cell? (Gortner, 1938, 548; Dobzhansky, 1951, 48; Beadle, 1948, 71). Or was it behaving as a virus? (Stanley, 1938, 491). On these alternatives our three authors would not be drawn. All they would say was:

> If the results of the present study on the chemical nature of the transforming principle are confirmed, then nucleic acids must be regarded as possessing biological specificity the chemical basis of which is as yet undetermined.
>
> (Avery, Macleod and McCarty, 1944, 155)

This was surely a case of sitting on the fence. Did Avery privately believe the transforming substance to be a gene? And if so why did he not say so? Hotchkiss has testified that Avery "was well aware of the implications of DNA transforming agents for genetics and infections" (1965, 6). We know that in May 1943 Avery wrote his brother a famous letter in which we find almost the identical phrase which cropped up in the '44 paper: "nucleic acids are not merely structurally important but functionally active substances in determining the biochemical activities and specific characteristics of cells," and he went on, "Sounds like a virus—may be a gene." Avery then added, as if hastily: "But with mechanisms I am not now concerned. One step at a time and the first step is, what is the chemical nature of the transforming principle? Someone else can work out the rest. Of course the problem bristles with implications." He went on to assure his brother that a lot of well documented evidence was needed before anyone could be convinced that protein-free DNA had the properties he claimed. In other words Avery deliberately concentrated his attention upon the chemical identity of the transforming substance and excluded other aspects.

One would still like to know just what Avery's private attitude was to the idea that transformation involved the incorporation of a gene into the recipient cell. MacLeod said: "He was not so much resistant to the idea as cautious. He was almost neurotic about overstating the case" (1968). It would be useless to expect that geneticists at the Rockefeller would have pushed this suggestion, for there were no geneticists at the laboratories and

hospital in New York. At the labs in Princeton there had been John Gowen, a pupil of T. H. Morgan, whose study of TMV mutagenesis we have noted, but he left Princeton in 1937 to become professor of genetics at Iowa State College and

> basic genetic investigations did not gain a solid foothold in either the New York or Princeton laboratories. Such studies, developed chiefly in zoological and botanical laboratories, apparently did not appeal to the administration as part of a programme then largely oriented toward pathology and physiology. Only years later, when gene action began to come within the grasp of biochemistry, was basic genetics to return to the Institute, under the leadership of Rollin D. Hotchkiss.
>
> (Corner, 1964, 309)

MacLeod recalled that since none of them was a geneticist "we all found ourselves reading genetic texts avidly" (MacLeod, 1968).

Dobzhansky's Interpretation

Sometime between 1940 and early 1942 Dobzhansky visited Avery's laboratory "and tried to argue that what were being observed were mutations like the mutations in *Drosophila.* Avery was slightly sceptical about it but said, 'I will look into the matter' " (Dobzhansky, 1968). About two weeks later Avery telephoned Dobzhansky and thanked him "for making an interesting suggestion that is probably what is taking place" (Dobzhansky, 1968). In the second edition of Dobzhansky's *Genetics and the Origin of Species,* the introduction to which is dated March 1941, there is the following statement:

> If this transformation is described as a genetic mutation—and it is difficult to avoid so describing it—we are dealing with authentic cases of induction of specific mutations by specific treatments—a feat which geneticists have vainly tried to accomplish in higher organisms.
>
> (Dobzhansky, 1941, 49)

Whether Avery really held this as the most likely explanation seems doubtful, but clearly he entertained the possibility seriously. Not until characteristics other than capsular polysaccharide were transferred in this way could the concept of directed mutagenesis be discarded. Harriet Taylor was later to be particularly forthright in rejecting the mutagenesis idea.

Dobzhansky was perhaps behaving as one would expect a geneticist to behave. Here, it seemed, was a mechanism for producing mutations, not just at random, but in a *predetermined* direction. He admitted that as a geneticist what interested him was that transformation involved a mutation; what it was produced by interested him much less (Dobzhansky, 1968). Likewise, Beadle in his Silliman lecture of 1948 put pneumococcal transformation alongside Auerbach's chemical mutagenesis and said:

> As a matter of fact, Pneumococcus type transformations, which appear to be guided in specific ways by highly polymerized nucleic acids, may well represent the first success in transmuting genes in predetermined ways.
>
> (Beadle, 1948, 71)

The three Rockefeller scientists therefore had good grounds for remaining non-committal, and when asked about this in 1966 MacLeod said: "at that time [1940s] all the genetic interpretations (plasmagenes, directed mutation, conversion, or Muller's pairing and crossing over) seemed plausible and they did not particularly favour one view" (Carlson, 1972).

Muller's Interpretation

At a conference held in New York in January 1946 Muller heard Delbrück report results for phages that seemed in principle like Avery's for pneumococcus, in that there was an apparent "conversion" of a viable type into a non-viable type of the sort with which it had been mixed. Now in Delbrück's experiments the rate of "conversion was so high as to discount the possibility of random mutation and selection, which Avery's results were really open to," and instead, Muller put forward the following idea:

> To my mind this suggests strongly that in both Delbrück's and Avery's cases what really happens is a kind of crossing over between chromosomes or protochromosomes of the inducer strain and those of the viable strain.
>
> (Muller, 1946)

It is well known that this suggestion has since been widely accepted. At the time of the conference it is said to have created quite an impression.

At the same conference Mirsky gave reasons for believing that the transforming substance contained chromosome material to which proteins were bound as in "chromosin". McCarty had tested the transforming activity of chromosin extracted from pneumococci by Mirsky and found it effective. Mirsky believed the thread-like bodies in his chromosin to be chromosomes or fragments of chromosomes. Muller was delighted with this suggestion because it brought the transformation process into the realm of the chromosome theory of the gene. No doubt it was Mirsky's paper at this New York meeting which suggested to Muller this chromosomal interpretation and he wrote:

> . . . we should have to suppose that these chromosomes can survive "extraction", that is, that they float more or less freely in the medium and can nevertheless, on coming into contact with the bacterial cell, enter into synapsis with homologous chromosome parts already there.
>
> (Muller, 1946)

Mirsky's Criticism

It is very understandable that Mirsky, who had devoted much of his time since 1942 to the chemistry of the nucleus, was critical of the efforts of three Doctors of Medicine to characterize the transforming substance of pneumococcus. Whilst the biochemists were showing how complex was the chemical constitution of chromosomes, Avery, MacLeod and McCarty were suggesting that a single substance could alone transfer biological specificity

from one cell to another. If they had identified this substance as a protein it would not have been so bad. But they made the revolutionary claim that it was a nucleic acid of the thymus type! They had, moreover, used a complex system which was inefficient (or so it appeared to be), unreliable and dependent upon competence factors whose chemical basis was unclear. As for the evidence from enzymology, Mirsky could point out that trypsin and chymotrypsin were inadequate as agents to destroy *all* types of protein. And in any case they only acted on proteins which had been partly or wholly denatured. Now pepsin is a general proteolytic enzyme, but this could not be used owing to the destructive effect of working at the required pH. (Here, pronase, had it been known at the time, could have filled the gap).

Apart from the evidence from enzymology there was the question of the purity of the DNA. Mirsky claimed that "pure, protein-free" nucleic acid could contain as much as 1 or 2 per cent of protein which histochemical tests would fail to reveal.

> One of the most sensitive direct tests for protein is the Millon reaction, but in our experience a nucleic acid preparation containing as much as 5 per cent of protein would give a negative Millon test. At present the best criterion for the purity of a nucleic acid preparation is its elementary composition and especially the nitrogen: phosphorus ratio. Presence of 2 per cent of protein would increase this ratio, but only by an amount that is well within the range of variation found for the purest nucleic acid preparations.
>
> (Mirsky and Pollister, 1946, 135)

Mirsky passed no comment on the evidence for purity of DNA from immunological tests, which Avery, MacLeod and McCarty claimed was capable of showing protein at a dilution of 1:50 000 (1944, 150). And even if this test was valid one could still claim that very little "genetic protein" was needed when so few cells in the recipient strain were transformed. On the other hand the transforming principle was active at a dilution of 1 in 6×10^8!

Nor was the purity of the transforming principle Mirsky's only concern. Following Alloway, Avery, MacLeod and McCarty had used sodium desoxycholate to liberate the nucleoprotein. But this substance acted as a detergent and was therefore likely to denature proteins. Mirsky, as we have seen, prepared "chromosin" from pneumococcal cells by his technique with molar NaCl. This procedure was less likely to denature the proteins and was therefore, he implied, preferable. Is this not carping over details? For as Mirsky himself admitted, the desoxycholate technique gave a higher yield of nucleic acid than the sodium chloride technique. And if residual protein was denatured by the detergent, then the conclusion that the transforming activity was associated with the nucleic acid and not with the protein was surely all the stronger. And was it not inconsistent for Mirsky to suggest in 1946 that desoxycholate denatures the protein in chromosin and in 1947 to discredit the evidence from the use of trypsin and chymotrypsin on the

grounds that these enzymes only act on denatured proteins? This looks suspiciously like a rearguard action fought by one who had backed the wrong horse!

Now it may be argued with some justice that Mirsky, in common with other biochemists, was merely adopting an empiricist stand, as when he said: " . . . it is not yet known which the transforming agent is—a nucleic acid or a nucleoprotein. To claim more, would be going beyond the experimental evidence" (1946, 135). In fact, it is doubtful that there is any such thing as a purely empiricist stand. Those like Mirsky, who preferred to withhold their support from Avery's conclusion, had very good reasons. These arose out of their knowledge of protein specificity and its chemical basis on the one hand, and the supposed lack of any chemical basis to specificity in the nucleic acids on the other. It can hardly be called empirical to go on *assuming* that nucleic acids lacked the required chemical sophistication for biological specificity, especially when evidence of immunological specificity had been forthcoming (pneumococcal antigenic RNA was species specific, as was TMV RNA). When Mirsky attended the Cold Spring Harbor Symposium on "Nucleic Acids and Nucleo-proteins" in June 1947 he came out with the same empiricist declaration: "In the present state of knowledge it would be going beyond the experimental facts to assert that the specific agent in transforming bacterial types is a desoxyribonucleic acid" (1947, 16). The French microbiologist, André Boivin, whose confirmation of Avery's work was the subject of Mirsky's criticism, conceded to Mirsky his empiricist statement but insisted that "the burden of the proof rests upon those who would postulate the existence of an active protein lodged in an inactive nucleic acid" (Boivin, 1947, 16). Looking back on those days of protein conservatism, Hotchkiss said he often wondered "which of our ideas take root merely because it becomes impracticable and then impolitic to take up the effort of questioning them!" (1966, 191). We have seen, also, that geneticists were chiefly interested in transformation as a case of mutation. Furthermore, when Muller gave the Messenger lectures in 1945 it was not evident that the acceptance of genetic specificity on the part of DNA *would deny it to proteins* and he said: "it may even be suspected . . . that for each different gene protein there is a special form of polymerized nucleic acid to match it" (1947a, 6). Before these lectures were published he had time to add a footnote about Mirsky's opinion that nucleic acids "do not constitute the main seat of the specificities" (*Ibid.*). After hearing Mirsky at New York in January 1946 he wrote to Darlington:

> Mirsky gave reasons for believing that Avery's so-called nucleic acid is probably nucleoprotein after all, with the protein too tightly bound to be detected by ordinary methods, and that what he had was free chromosomes, or pieces of chromosomes. This protein is a higher protein, like Stedman's chromosomin, in which Mirsky now believes, the histone and protamine being easily removed while the higher protein is left attached to the

> nucleic acid. All this too seems to me to fit into the same picture, and I am trying to induce Mirsky to bring along some of his and of Avery's extracted material to be looked at by Mrs. Baylor under the electron microscope at the University of Illinois.
>
> (Muller, 1946)

Muller inserted a very similar passage in a footnote to his Pilgrim Trust Lecture of 1946 before its publication (1947b, 23), and he ended the address as follows:

> Thus it may be that nucleic acid in polymerized form provides a way of directing such a flow of energy into specific complex patterns of gene building or for gene reactions upon the cell. But to what extent the given specificity depends on the nucleic acid polymer itself, rather than upon the protein with which it is ordinarily bound, must as yet be regarded as an open question.
>
> (Muller, 1947b, 24)

It may well have been Muller's acceptance of Stanley's initial identification of crystalline TMV as a protein which had already set his view on the chemistry of the gene (Carlson, 1966, 128). Whilst this may be true of Muller in the late '30s, I doubt it can be held responsible for his view in 1946, when Mirsky's authority seems to have been the major influence. Through Muller's widely read Pilgrim Lecture, this influence was spread to a wide audience. How should we evaluate this influence today? Was it beneficial or harmful? Clearly it was positive in the sense that more evidence was called for. On the other hand it protected the Protein Version of the Central Dogma on the grounds that the task of demonstrating the specificity of DNA had yet to be achieved.